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(71) Applicant (for all designated States except US): COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION [AU/AU]; 14 Limestone Avenue, Campbell, ACT 2601 (AU).			
(72) Inventors; and			
(75) Inventors/Applicants (for US only) : REILLY, Wayne, Gerard [AU/AU]; 72 Briens Road, Northmead, NSW 2152 (AU). WHITTAKER, Robert, George [AU/AU]; 23 Ramsay Avenue, West Pymble, NSW 2073 (AU). JENNINGS, Philip, Anthony [AU/AU]; Commonwealth Scientific and Industrial Research Organisation, Division of Biomolecular Engineering, 103 Delhi Road, North Ryde, NSW 2113 (GB). FINNEY, Kenneth, Geoffrey [AU/AU]; 32 Willow Crescent, Ryde, NSW 2112 (AU).			

**(54) Title:** SELF-ADJUVANTING PEPTIDE VACCINE DELIVERY SYSTEM AND PRODUCTION THEREOF**(57) Abstract**

The present invention provides self-adjuvanting vaccines for use in raising antibodies to peptides without the use of oil or alum adjuvants. Further, the present invention provides methods of therapy using these vaccines and has particular application in chemical castration. In one aspect the vaccine comprises in admixture a peptide conjugated to 1-3 fatty acids and a peptide conjugated to a carrier protein. In preferred forms the peptide is conjugated to the fatty acids via a tromethamine or ethanolamine derivative. The preferred protein carrier is Type 4 fimbriae.

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SELF-ADJUVANTING PEPTIDE VACCINE DELIVERY  
SYSTEM AND PRODUCTION THEREOF

Background of the Invention

The present invention relates generally to 5 self-adjuvanting vaccines for use in raising antibodies to peptides without the use of oil or alum adjuvants. The present invention further relates to the use of this vaccine and to methods of therapy. The present invention has particular application in chemical castration.

10 The use of leutinising hormone releasing hormone (LHRH) in chemical sterilisation of both male and female animals is known. In prior art techniques animals are immunised with preparations including LHRH and this immunisation leads to dysfunction of the gonads and 15 consequent induced sterility. In male animals reduction in testicular size relative to untreated controls may be clearly seen. Unfortunately, the mere immunisation with LHRH is not a totally effective method of chemical castration. Typically, whilst in the majority of one 20 group of animals the immunisation with LHRH will have the desired effect, the immunisation will have little effect on a number of animals in that sample. As will be readily appreciated this is a significant drawback because, for example, if one unaffected male remains in the immunised 25 herd this male may fertilise a number of females within the herd. For this reason chemical sterilisation utilising LHRH has to date achieved only limited application, with physical and other methods of sterilisation still predominating.

30 In the present applicant's copending Australian patent application No 17049/88 there is disclosed an effective method of peptide production by protein engineering. This method involves the culturing of genetically engineered bacteria which produce, as 35 extracellular structures, Type 4 fimbriae, the peptide

being produced in association with these fimbriae. This method comprises culturing bacteria containing the gene encoding the fimbrial subunit of Type 4 fimbriae to which has been added at the C-terminal end the nucleic acid sequence encoding the desired peptide. In further work on this system the present inventors have found that not only does this method provide a simple and efficient means of producing a peptide but that the fimbrial protein acts as a powerful immunoadjuvant.

10 The present inventors have also produced a peptide having a sequence derived from LHRH in association with Type 4 fimbriae. It has been found that this peptide conjugated to bacterial Type 4 fimbriae provides a very effective chemical sterilisation agent.

15 In the present applicant's copending International Patent Application No PCT/AU90/00599 (the disclosure of which is incorporated herein by reference) there is disclosure of means of linking fatty acids and the like to peptides.

20 The present inventors have also found that co-administration of a peptide/fatty acid conjugate with a peptide/carrier protein (such as fimbrial protein) conjugate yields a heightened antibody response to the peptide.

25 In addition the present inventors have found that the antibody response in an animal can be enhanced by the use of the fimbrial protein of a Type 4 fimbriate bacteria.

#### Summary of the Invention

Accordingly, in a first aspect the present invention 30 consists in a vaccine for use in raising an immune response to a peptide, the vaccine comprising in admixture the peptide conjugated to 1 to 3 fatty acids and the peptide conjugated to a carrier protein.

35 In a preferred embodiment of the first aspect of the present invention the peptide has or includes one of the

following amino acid sequences:-

SGGWSYGLRPGG;

WSYGLRP;

WSYGWLW; or

5 WSYGLQP.

In a second aspect the present invention consists in a vaccine for use in chemical sterilisation the vaccine comprising a peptide which has or includes an amino acid sequence selected from the group consisting of:-

10 SGGWSYGLRPGG, WSYGLRP, WSYGWLP and WSYLLQF, the peptide being conjugated either to 1 to 3 fatty acids or to a fimbrial subunit.

In a preferred embodiment of the invention the carrier protein is a fimbrial sub unit protein, ovalbumin, 15 bovine serum albumin, tetanus toxin, or keyhole limpet haemocyanin. It is presently preferred, however, that the carrier protein is a fimbrial protein subunit and that the subunit proteins are assembled into fimbriae. It is most preferred that the fimbriae are Type 4 fimbriae.

20 The peptide is preferably conjugated to 1 to 3 fatty acids via a tromethamine or an ethanolamine derivative.

It is presently preferred that the peptide is linked to three fatty acids and more preferably that each are the same fatty acid. It is also preferred that the fatty acid 25 has a carbon chain of 3 to 18 carbon atoms and most preferably 16 carbon atoms.

The present inventors have also found that partial denaturation of the peptide/fimbrial protein carrier results in a higher antigenic response. This partial 30 denaturation is preferably obtained by treatment of the peptide/carrier protein at a pH of less than or equal to 4 and preferably at a pH of about 1. Given the enhanced response that this partial denaturation provides in a preferred form of the invention the peptide/fimbrial 35 protein conjugate is subjected to a a partial denaturation

prior to admixture with the peptide/fatty acid conjugate.

As will be appreciated by those skilled in the art while any peptide can be used in the vaccine of the first aspect of the present invention the preferred peptides are

5 LHRH derived peptides. Where the peptide is an LHRH peptide, or a derivative, the vaccine may be used for chemical sterilization of animals or for use as an alternative to the present LHRH agonist and antagonist therapy for human sex hormone dependent cancers. Four of  
10 the most commonly occurring human sex hormone dependent cancers are prostate, breast, endometrial and ovarian cancer. These conditions may be susceptible to treatment by vaccination with the vaccine of the present invention using the LHRH peptide or derivative. The vaccine of the  
15 present invention could also be used as an adjunct to the present LHRH agonist and antagonist therapy for these diseases.

It will be appreciated by those skilled in the art that a number of modifications may be made to the peptides  
20 preferably used in the present invention without deleteriously effecting the biological activity of the peptide. This may be achieved by various changes, such as insertions, deletions and substitutions, either conservative or non-conservative in the peptide sequence  
25 where such changes do not substantially alter the nature of the immune response raised by the peptide. By conservative substitutions the intended combinations are:-

G,A; V,I,L,M; D,E; N,Q; S,T; K,R,H; and F,Y,W.

The vaccine of the first aspect of the present  
30 invention will also have applicability in the treatment of diseases such as AIDS, malaria, influenza, zone pellucida peptide epitopes or hepatitis where peptide epitopes have been identified and a vaccine approach is possible.

The vaccine of the first aspect of the present  
35 invention comprising an admixture of peptide/fatty acid.

conjugate with peptide/carrier protein conjugate when used without oil or alum adjuvants induces high level antibody responses in animals. The ability of this vaccine to induce such high level response without requiring oil or 5 alum based adjuvants is of particular benefit. Such findings will allow the use of this vaccine where the use of oil based adjuvants is not permitted and the use of alum based adjuvants is being questioned, e.g., human vaccines or vaccines for companion animals.

10 The preferred form of preparing the peptide/type 4 fimbrial protein conjugate involves using the method disclosed in Australian patent application No 17049/88. By using this method the desired peptide can be expressed 15 in association with Type 4 fimbriae by genetically engineered strains of *Pseudomonas*. This method is, however, to some extent, limited in that the peptide epitope size that can be incorporated into the fimbriae is dependent on the degree of modification which can be made before the fimbriae is no longer properly assembled.

20 Investigations have shown that peptides of up to approximately 20 amino acid residues can be produced in association with the fimbriae in *Pseudomonas aeruginosa*. Accordingly, it is presently preferred that the peptides are able to be expressed in association with fimbriae in 25 *Pseudomonas aeruginosa*. If, however, the peptide epitope is too large or cannot be expressed in this fimbrial system it is still possible to use the vaccine of the present invention by simply chemically conjugating the peptide with the fimbrial protein.

30 The response to the vaccine component of the present invention may also be enhanced by the use of T-cell, H<sub>2</sub> receptor antagonists, e.g., cimetidine or carnosine, or other immunomodulators e.g., cytokines or immunostimulatory peptides which may act as immunomodulators to overcome 35 carrier protein unresponsiveness or to further enhance the

immune system.

The present inventors also believe that the antibody response in an animal to an antigen may be heightened by the use of fimbrial protein of a Type 4 fimbriate bacteria.

5 Accordingly, in a third aspect the present invention consists in a method of enhancing the antibody response to an antigen in an animal, the method comprising to the animal an effective amount of the antigen conjugated to the fimbrial protein of a Type 4 fimbriate bacteria.

10 As is disclosed in application No PCT/AU90/00599 a wide range of fatty acids may be linked to the peptide via a tromethamine or ethanolamine derivative. In addition, 1-3 fatty acids may be linked to the peptide. It should also be noted that the peptide linked to the fatty acids 15 can be of virtually unlimited size.

In order that the nature of the present invention may be more clearly understood a preferred form thereof will now be described with reference to the following examples and figures in which:-

20 Figs. 1-4 show the results of a comparative vaccination trial using ten animals/group, mice were given two vaccinations at four week intervals and data collected four weeks after the second vaccination; (Fig. 1 control, Fig 2 100 $\mu$ g untreated LHRH-fimbriae and Freund's 25 Incomplete Adjuvant (FIA), Fig. 3 Wild Type Fimbriae, Fig. 4 untreated LHRH-Fimbriae admixture with LHRH ala-tris-tripalmitate (ATP3) conjugate;

30 Fig. 5 shows the antibody response for the animals set out in Figs. 2 and 4, the light hatch represents the serum LHRH antibody levels for group in Fig 2 and the dark hatch group Fig 4. Groups Fig. 1 and Fig. 3 had no LHRH antibodies;

Fig. 6 shows the results of admixture vaccinations using LHRH-acid treated fimbriae or LHRH-ovalbumin 35 conjugate with the ATP3 conjugate. Four groups of ten

mice were given a primary vaccination followed by a secondary vaccination four weeks later.

6(a) mice vaccinated with acid treated LHRH-fimbriae adjuvanted with FIA (ATF-FIA);

5 6(b) mice vaccinated with acid treated LHRH-fimbriae in admixture with ATP3 conjugate (ATF-ATP3);

6(c) mice vaccinated with LHRH-ovalbumin conjugate adjuvanted with FIA (OV-FIA); and

10 6(d) mice vaccinated with LHRH-ovalbumin conjugate in admixture with ATP3 conjugate (OV-ATP3). Testes weights are presented for each individual animal;

Fig. 7 shows the antibody results from the vaccinations set out in Fig. 6 □ ATF-FIA; ▨ ATF-ATP3; ■ OV-FIA; and ▨ OV-ATP3.

15 Fig. 8 shows antibody results obtained in immunocastrated mice after regeneration of testes were revaccinated with untreated LHRH-fimbriae and FIA;

□ prior to revaccination, ▨ one week after revaccination, ■ nine weeks after revaccination. The 20 background absorbance level for this data was 0.19 units;

Fig. 9 shows the results of mouse vaccination trials using acid treated LHRH-fimbriae adjuvanted with FIA and potentiated with cimetidine (100 $\mu$ l of 5mg/ml given every second day for 6 days after vaccination). The mice were 25 vaccinated with 100 $\mu$ g of fimbrial protein given subcutaneously and revaccinated four weeks later. The testes weights and LHRH antibody levels for each individual mouse are shown in Figs. 9a and 9b respectively;

Fig. 10 shows the results of mouse vaccination trials 30 using acid treated LHRH-fimbriae, immunopotentiated with carnosine (100 $\mu$ l of 5mg/ml given every second day for 6 days after vaccination) and adjuvanted with FIA. The mice were vaccinated with 100 $\mu$ g of fimbrial protein given subcutaneously and revaccinated four weeks later. The 35 testes weights and LHRH antibody levels for each

individual mouse are shown in Figs 10a and 10b respectively;

Fig. 11 shows the results of a mouse vaccination trial using acid treated LHRH-fimbriae in a water/glycerol mixture and not adjuvanted with oil or alum. The mice were vaccinated with 150mg of protein subcutaneously and given a booster vaccination four weeks later. The testes weights and LHRH antibody levels for each individual mouse four weeks after the booster vaccination are shown in Figs 11a and 11b respectively; and

Fig. 12 shows the results of mouse vaccination trials using acid treated LHRH-fimbriae in admixture with LHRH-ATP3 and immunopotentiated with either cimetidine or carnosine. The mice were given a primary vaccination with 100mg acid treated LHRH-fimbriae together with 2mg of LHRH-ATP3 and immunopotentiated with three doses of 500mg of either cimetidine or carnosine. A secondary vaccination was given four weeks later, the testes weights and LHRH antibody levels for each individual mouse eight weeks after the primary vaccination is shown in Figs 12a (□ cimetidine, ■ carnosine) and 12b (□ cimetidine, ■ carnosine) respectively;

#### Materials and Methods

##### plasmids and bacterial strains

The multifunctional vector pFEM2 was constructed and used for oligonucleotide-directed mutagenesis of the fimbrial subunit gene and for the testing of the fimbrial expression of modified subunits in *P.aeruginosa*. In brief, the plasmid contains a c1857 gene cartridge obtained from pC121 and a fimbrial subunit expression cartridge from pJSM129. These were recombined to give a vector which can replicate in *E.coli* and *P.aeruginosa*, has a M13 intergenic region for the production of single stranded DNA and carries a *Dichelobacter nodosus* Al serotype fimbrial subunit expression cartridge. Regulated

expression of the fimbrial subunit is possible because of the plasmid-borne c1857 gene. The P.aeruginosa strain K/2Pfs was used for fimbrial subunit expression. Competent cells were prepared using the procedure 5 described for P.aeruginosa. Transformed cells were propagated at 37°C or at 42°C when fimbrial subunit expression was desired. P.aeruginosa transformants were cultured in Trypticase Soy Broth (TSB, 30 gms/litre) containing 0.15 mg/ml carbenicillin.

10 Expression of Fimbrial Subunit Bearing the LHRH Graft  
Oligoneucleotide directed mutagenesis and hybridisation screening was used to insert the LHRH related peptide into the pFEM/2 plasmid. DNA sequence analysis of a plasmid designated pMF2/1, isolated from one 15 of these clones confirmed that the desired peptide had been grafted to the fimbrial subunit. pMF2/1 was used to transform P.aeruginosa K2 and expression of the modified subunit tested. A protein with a molecular weight greater than that of the unmodified fimbrial subunit was induced 20 and could be detected extracellularly. The extracellular material was immunologically identified as D.nodosus fimbrial subunit and electron microscope examination revealed that the material existed as intact fimbriae. These results showed that fimbrial subunit bearing the 25 LHRH graft was assembled and harvested as morphogenetically correct, extracellular structures.

Induction of Fimbrial Subunit Expression

For the induction of fimbriae in P.aeruginosa, colonies from 37°C overnight culture plates were mixed 30 into 8ml portions of Trypticase Soy Broth (TSB) and 2ml aliquots spread onto 245mm x 245mm TSB agar plates containing 0.15mg/ml of Carbenicillin. Plates were incubated at 42°C for 18 to 24 hr and cells harvested by scraping into a minimal volume of phosphate-buffered 35 saline. Fimbriae were precipitated from cell-free

supernatents of the harvested cells in the presence of 0.1M magnesium chloride. Whole fimbrial samples were than analyzed for the protein content of samples using a dye-binding procedure BCA (Pierce, USA) and samples taken 5 for the electrophoretic analysis using a urea-SDS-polyacrylamide gel system.

Acid Treatment of PAK2 Fimbriae

Fimbrial preparations after  $MgCl_2$  precipitation were washed with distilled water and resuspended at a 10 concentration of 10mg/ml. The pH of the fimbrial solution was adjusted to a pH of 1 using AR grade  $H_3PO_4$  (85% w/v, Ajax Chemicals). The pH adjusted solution was then left undisturbed at  $40^{\circ}C$  for 16 hours. The acid treated fimbriae were then neutralised with 100 $\mu$ l of 10 M NaOH 15 and examined using non denaturing PAGE. The protein concentration was determined using a BCA method (Pierce, USA) and then stored at  $-20^{\circ}C$  until required.

Determination of LHRH antibody levels using ELISA.

Samples of blood were taken from mice at death and 20 the sera from each stored at  $-20^{\circ}C$  until the antibody level was determined. A 200 $\mu$ l aliquot of gelatin conjugated LHRH at  $4^{\circ}C$ . These were then washed twice with phosphate buffered saline containing 0.5ml of Tween 20/litre (PBS-Tween) and blocked for 1.5 hours with 0.5 25 gms/100 ml gelatin. The plates were washed twice and 200 $\mu$ l of 1:2500 mouse sera, from the experimental and control mice, added and left for 1.5 hours. These were again washed and a 1:2000 dilution of (horse radish peroxidase) HRP conjugated rabbit anti-mouse IgG (Dako 30 Corp., Denmark) added to each. These were left for 1.5 hours washed 3 times with PBS-Tween and treated with OPD to determine the response. The absorbances were measured at 492 nm using a plate reader.

Immunization of Mice.

35 Twenty-five-day-old male Swiss/Balb c mice were

immunized intraperitoneal (I.P.) with 100 $\mu$ g to 150  $\mu$ g of LHRH engineered PAK2 fimbriae in sterile distilled water at 1:1 (50 $\mu$ l : 50 $\mu$ l) mixture with Freund's Incomplete Adjuvant (FIA) (Difco, UK) or Titremax (CytRx Corp, USA). A secondary immunisation was given at one month and the animals were sacrificed at two months after the primary immunisation. Blood and testes samples were taken at this point and used for LHRH antibody determination and statistical analysis.

10 Preparation of Ala-T Mono, Di and Tri Palmitate

1 miliequivalent of LHRH peptide was reacted with 3 miliequivalents of O(N-succinimidyl)-N,N,N',N' - tetramethyl uronium tetrafluoro borate (Fluka, 85975) in dimethyl formamide and N-ethyl-di-isopropyl amine was 15 added to the apparent pH of 8.7. Active ester was formed in excess of 75% yield. To this solution 2 miliequivalents of ala-tris(palmitate, dipalmitate or tripalmitate) was added in dichloromethane (2-3ml) and stirred for 1 hour. The apparent pH was readjusted to 8.5.

20 The reaction was monitored by HPLC using mobile phase consisting of

- (i) Buffer A - 0.1% TFA : acetonitrile, 50:50, and
- (ii) Buffer B - acetonitrile: THF, 50:50 using a Novapak C4 column.

25 Purification of the products was by semi-preparative HPLC, on a C4 column and after the removal of solvents lyophilised from t-butyl alcohol.

Results

In order to assess the effectiveness of the LHRH 30 related peptide/fimbrial protein and peptide/fatty acid conjugates mice were immunised with a variety of vaccine combinations. Figures 1-4 shows a comparative vaccination trial using 10 animals per group. Mice were given two vaccinations at a four week interval and data collected 35 four weeks after the second. The control animals (Fig. 1)

and animals which received wild type fimbriae (fimbriae containing no peptide additions) (Fig. 3) showed no reduction in testes weight. (Testes weight of less than 0.25g is considered a positive response) In contrast the 5 animals vaccinated with acid treated LHRH related peptide/fimbrial protein with Freund's Incomplete Adjuvant (FIA) showed a marked reduction in testes weight. Similarly, mice receiving untreated LHRH related peptide/fimbrial protein in admixture with peptide/fatty acid conjugate showed a marked reduction in testes 10 weight. Figure 5 shows the antibody response in these animals.

Figure 6 shows the results of mouse vaccinations with LHRH fimbrial protein conjugate and LHRH-ovalbumin conjugate with ATP3 fatty acid conjugate (ala-tris-tripalmitate). Groups of 10 mice were 15 vaccinated twice at four week intervals with combinations of the ATP3 conjugate and either LHRH conjugated to ovalbumin or LHRH conjugated to acid treated fimbriae. 20 Data was collected four weeks after the second vaccination. The positive Control for the data set was LHRH acid treated fimbriae or LHRH-ovalbumin conjugate with FIA. Both LHRH presenting carrier molecules in combination with the fatty acid peptide conjugate yielded 25 good physical responses, i.e., gonadal atrophy. The antibody responses for these animals are presented in Figure 7.

To exemplify the vaccines reversibility, five mice which had been vaccinated with LHRH-related 30 peptide/fimbrial protein and (FIA) were left to recover after gonadal atrophy had occurred, i.e. 120 days after the primary vaccination. These mice were revaccinated with LHRH-fimbriae and 14 days later they were once again displaying marked gonadal atrophy. This indicates that 35 the castration effect induced by the LHRH/fimbrial antigen

is reversible and can be maintained with subsequent vaccinations. Antibody levels for these mice one week and four weeks after vaccination are presented in Figure 8.

5 Figs. 9 and 10 show that the antibody response and reduction in testes weight can be increased by using the immunopotentiating agents cimetidine and carnosine.

10 Fig. 11 shows that significant reductions in testes weight and increases in antibody levels can be obtained by administering acid treated LHRH-fimbriae conjugate in the absence of an adjuvanting agent. This ability to enhance 15 antibody response using the Type 4 fimbriae is unexpected. It is believed that this unexpected result opens up the possibility of enhancing antibody responses by using Type 4 fimbriae as a "self-adjuvanting" carrier for peptides conjugated to the fimbriae.

20 Fig. 12 shows that significant reductions in testes weight and high antibody levels are obtained in animals vaccinated with acid treated LHRH-fimbriae in conjunction with LHRH-ATP3. It is of particular note that these reductions in testes weight and antibody levels were obtain without the use of oil or alum based adjuvants.

25 This "self-adjuvanting" ability of the vaccine admixture is of particular relevance given the inability to use oil based adjuvants in humans and the questions being raised concerning the use of alum based adjuvants.

30 As will be seen the present invention provides a vaccine which can be used to raise significant antibody levels against peptides without the use of oil or alum based adjuvants. This discovery is of great utility given the present concern regarding the use of oil and alum based adjuvants. Further, these findings open a new avenue for vaccines for human use.

35 It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments

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without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

## CLAIMS:-

1. A vaccine for use in raising antibodies to a peptide, the vaccine comprising in admixture the peptide conjugated to 1 to 3 fatty acids and the peptide conjugated to a carrier protein.
- 5 2. A vaccine as claimed in claim 1 in which the carrier protein is selected from the group consisting of fimbrial subunit protein, ovalbumin, bovine serum albumin, tetanus toxin and keyhole limpet haemocyanin.
- 10 3. A vaccine as claimed in claim 1 or claim 2 in which the carrier protein are fimbrial subunit proteins assembled into mature fimbriae.
4. A vaccine as claimed in claim 3 in which the fimbrial subunit protein is Type 4 fimbriae.
- 15 5. A vaccine as claimed in any one of claims 1 to 4 in which the peptide is conjugated to 1 to 3 fatty acids via a tromethamine derivative or an ethanolamine derivative.
6. A vaccine as claimed in any one of claims 1 to 4 in which the peptide is selected from the group consisting of
- 20 LHRH peptides, AIDS peptide epitopes, malaria peptide epitopes, influenza peptide epitopes and hepatitis peptide epitopes and zona pellucida peptide epitopes.
7. A vaccine as claimed in claim 6 in which the LHRH peptide is selected from the group consisting of
- 25 SGGWSYGLRPGG, WSYGLRP, WSYGWLP and WSYGLQP.
8. A vaccine for use in chemical sterilisation, the vaccine comprising a peptide which has or includes an amino acid selected from the group consisting of SGGWSYGLRPGG, WSYGLRP, WSYGWLP and WSYGLQP, the peptide being conjugated either to 1 to 3 fatty acids or a fimbrial subunit protein.
- 30 9. A vaccine as claimed in claim 8 in which the fimbrial subunit proteins are assembled into mature fimbriae.
10. A vaccine as claimed in claim 9 in which the fimbrial subunit protein is Type 4 fimbriae.
- 35

11. A vaccine as claimed in any one of claims 8 to 10 in which the peptide is conjugated to 1 to 3 fatty acids via a tromethamine derivative or an ethanolamine derivative.
12. A vaccine as claimed in any one of claims 3 to 11 in 5 which the peptide/fimbrial protein conjugate is exposed to an acid treatment at pH less than or equal to 4.
13. A vaccine as claimed in claim 12 in which the peptide/fimbrial protein is exposed to an acid treatment at pH 1.
- 10 14. A vaccine as claimed in any one of claims 1 to 13 in which the peptide is linked to 3 fatty acids each of which is the same fatty acid.
15. A vaccine as claimed in any one of claims 1 to 14 in which the fatty acid has a carbon chain of 3 to 18 carbon atoms.
16. A vaccine as claimed in claim 15 in which the fatty acid has a carbon chain of 16 carbon atoms.
17. A vaccine as claimed in any one of claims 1 to 16 in which the vaccine further includes cimetidine, carnosine, 20 cytokines or immunostimulatory peptides.
18. A method of chemically sterilising an animal comprising administering to the animal a vaccine as claimed in any one of claims 3 to 17.
19. A method of treating a subject suffering from 25 prostate cancer, breast cancer, endometrial cancer or ovarian cancer comprising administering to the subject a vaccine as claimed in any one of claims 3 to 17.
20. A method of enhancing the antibody response to an antigen in an animal, the method comprising administering 30 to the animal an effective amount of the antigen conjugated to the fimbrial protein of a Type 4 fimbriate bacteria.
21. A method as claimed in claim 20 in which the antigen is a peptide selected from the group consisting of 35 SGGWSYGLRPGG, WSYGLRP, WSYGWLP and WSYGLQP.

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22. A method as claimed in claim 20 or claim 21 in which fimbrial protein is exposed to an acid treatment at pH less than or equal to 4.

23. A method as claimed in claim 22 in which the fimbrial 5 protein is exposed to an acid treatment at pH 1.

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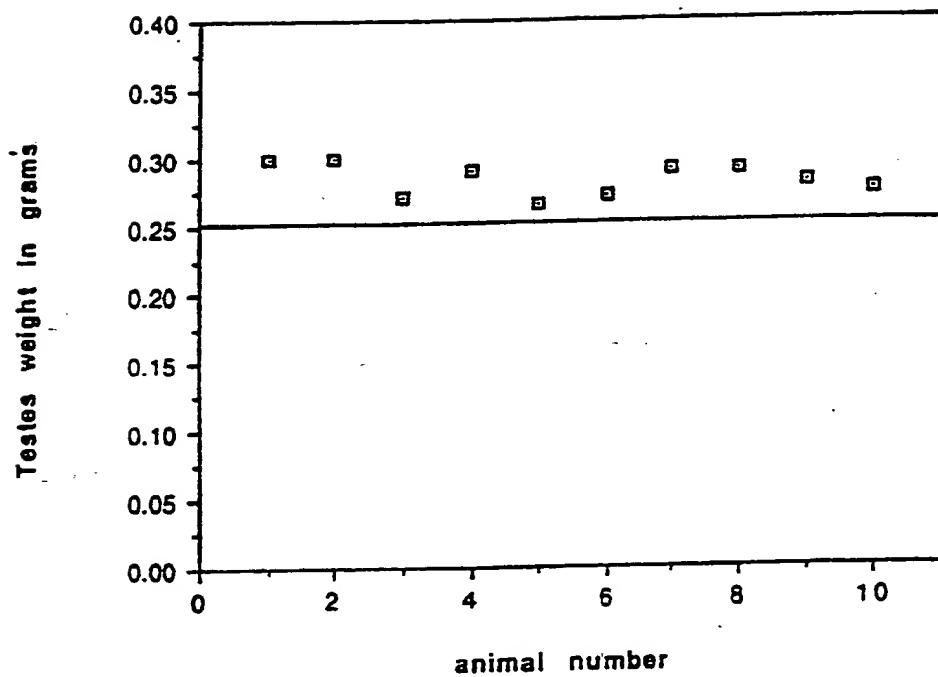


FIG. 1

2/17

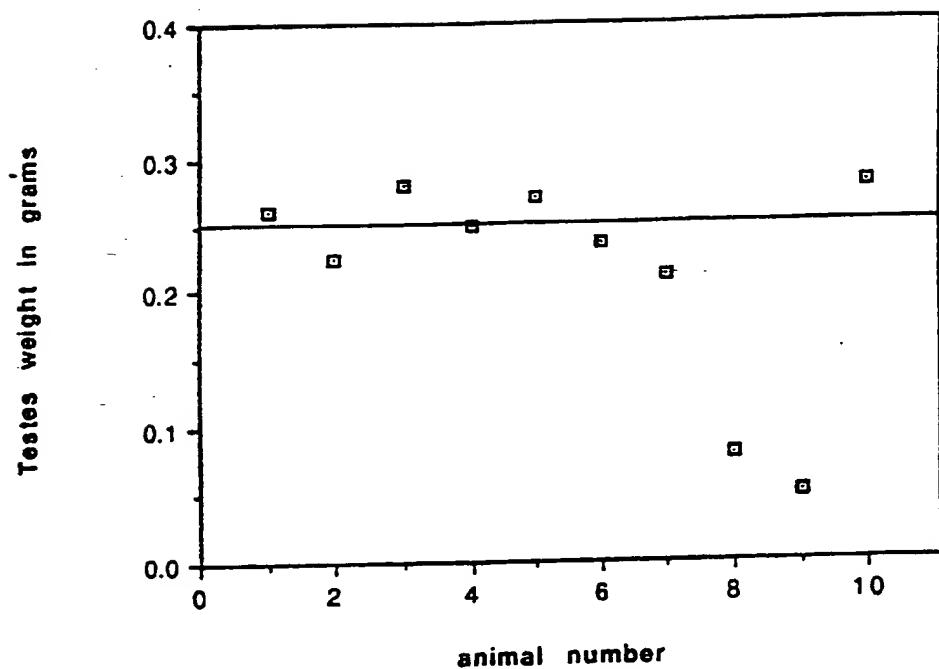


FIG. 2

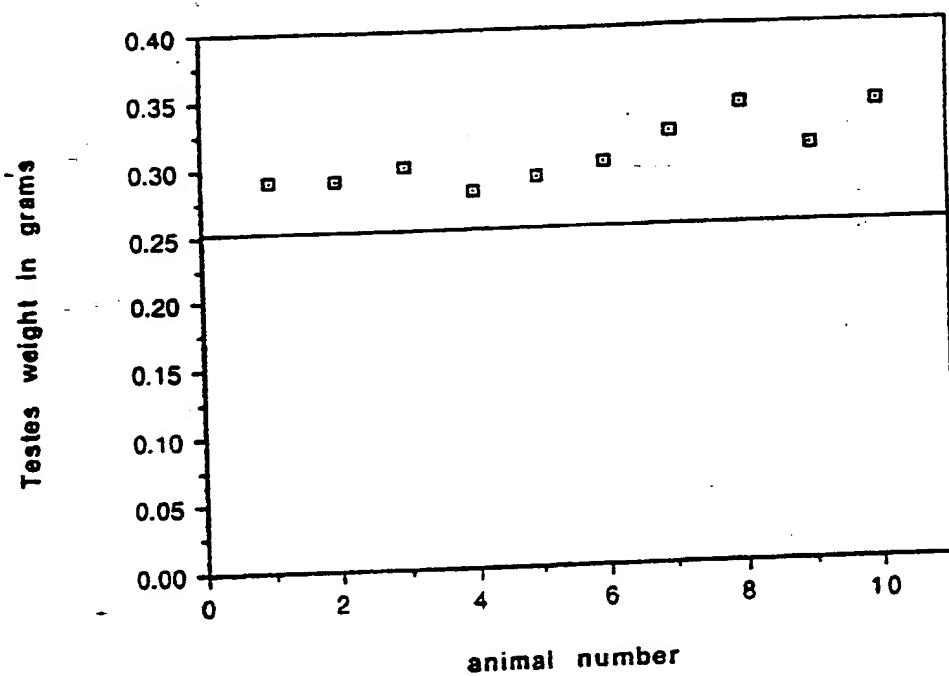


FIG. 3

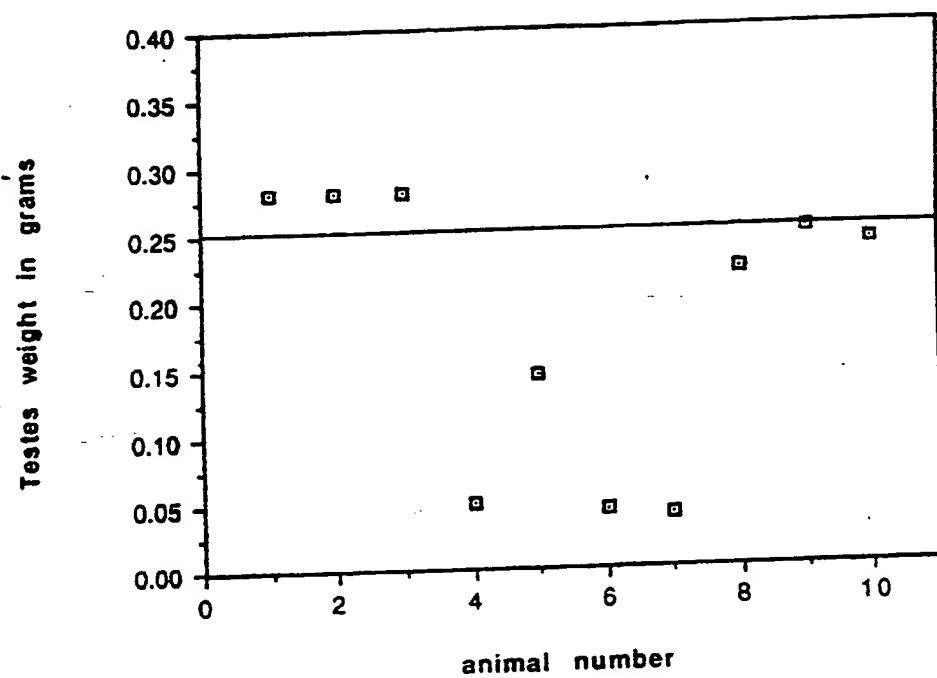


FIG. 4

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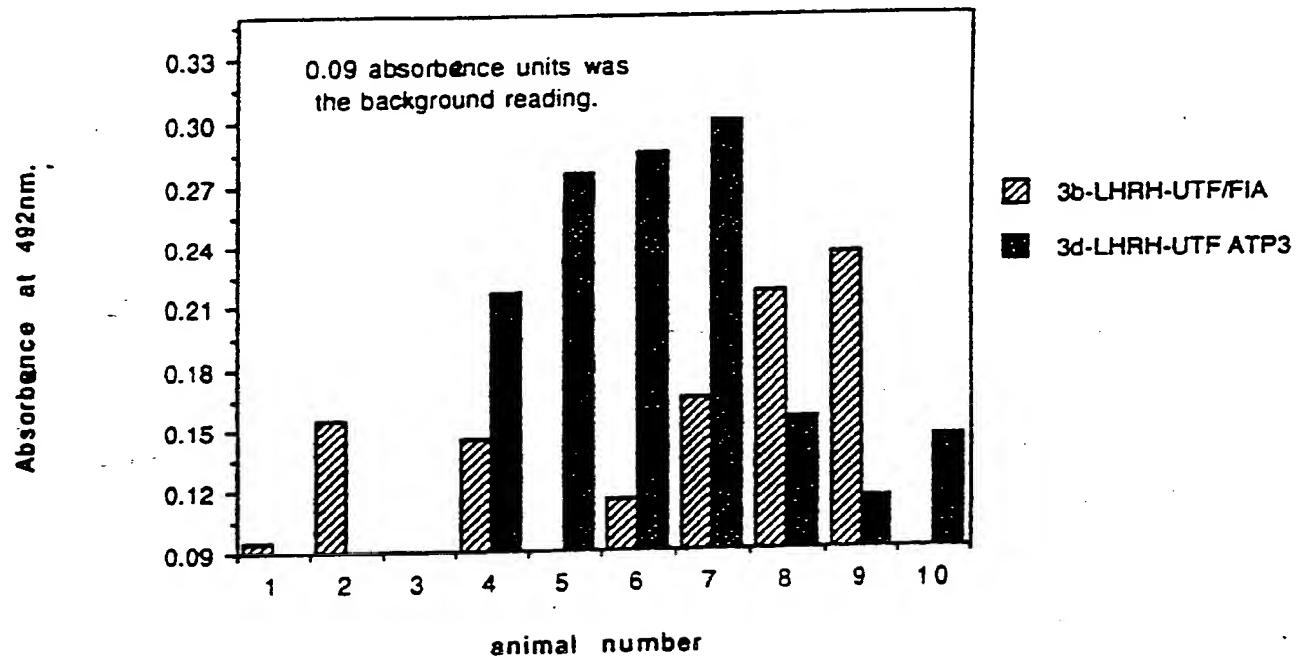


FIG. 5

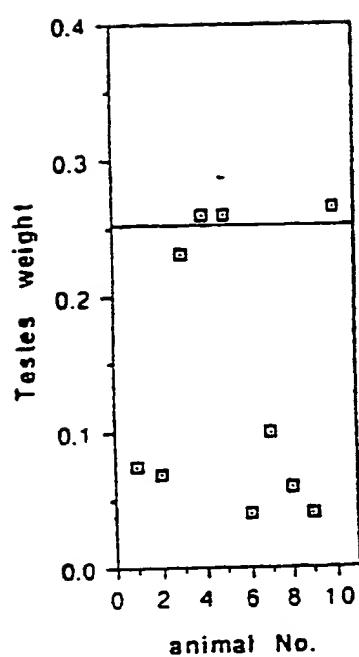
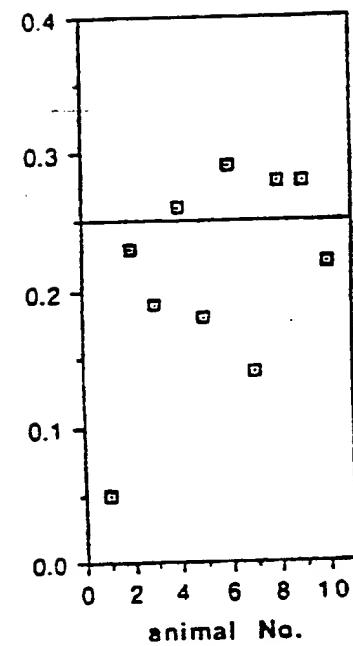
**a****b**

FIG. 6a

FIG. 6b

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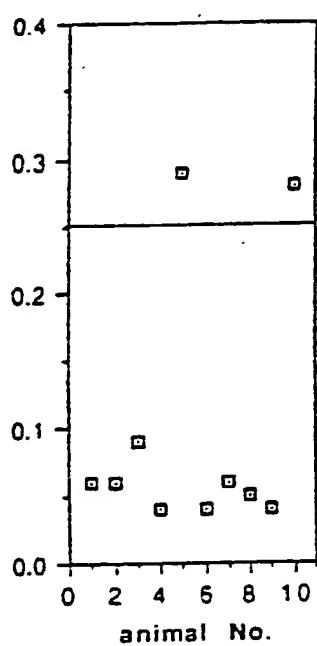
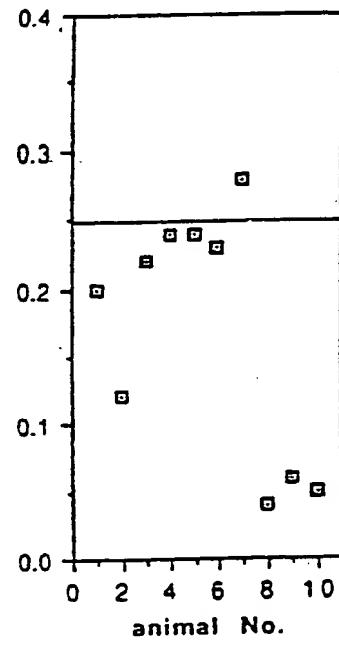
**c****d**

FIG. 6c

FIG. 6d

Data from vaccinations with ATP3 fatty acid with acid treated LHRH fimbriae (ATF) or LHRH ovalbumin (OV).

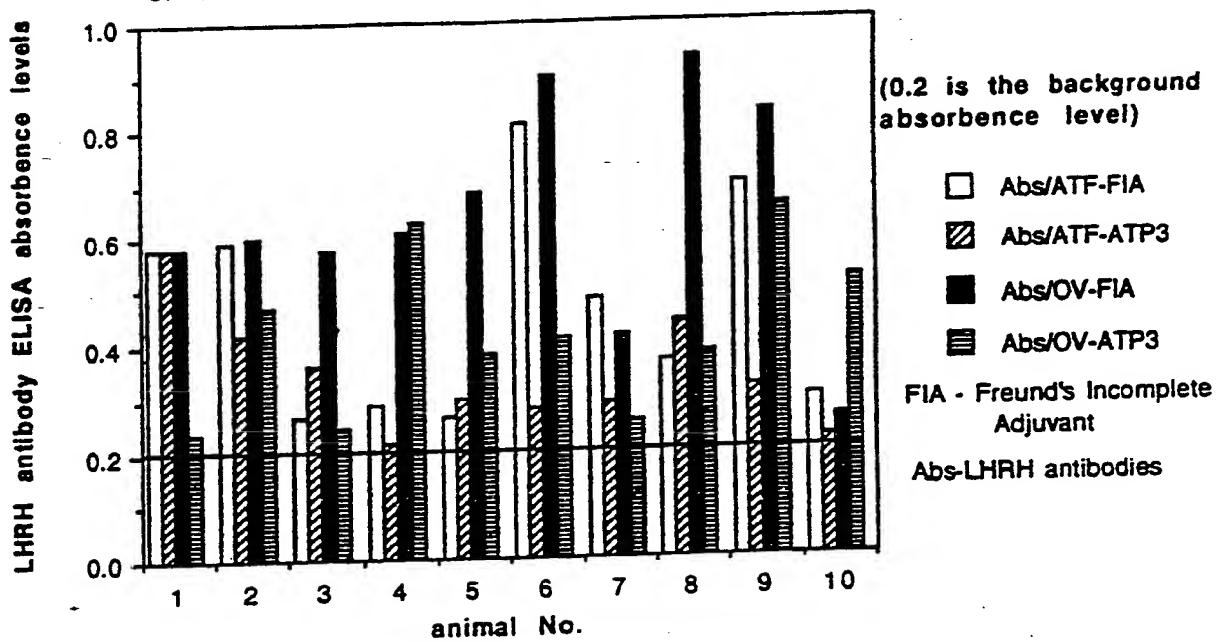


FIG. 7

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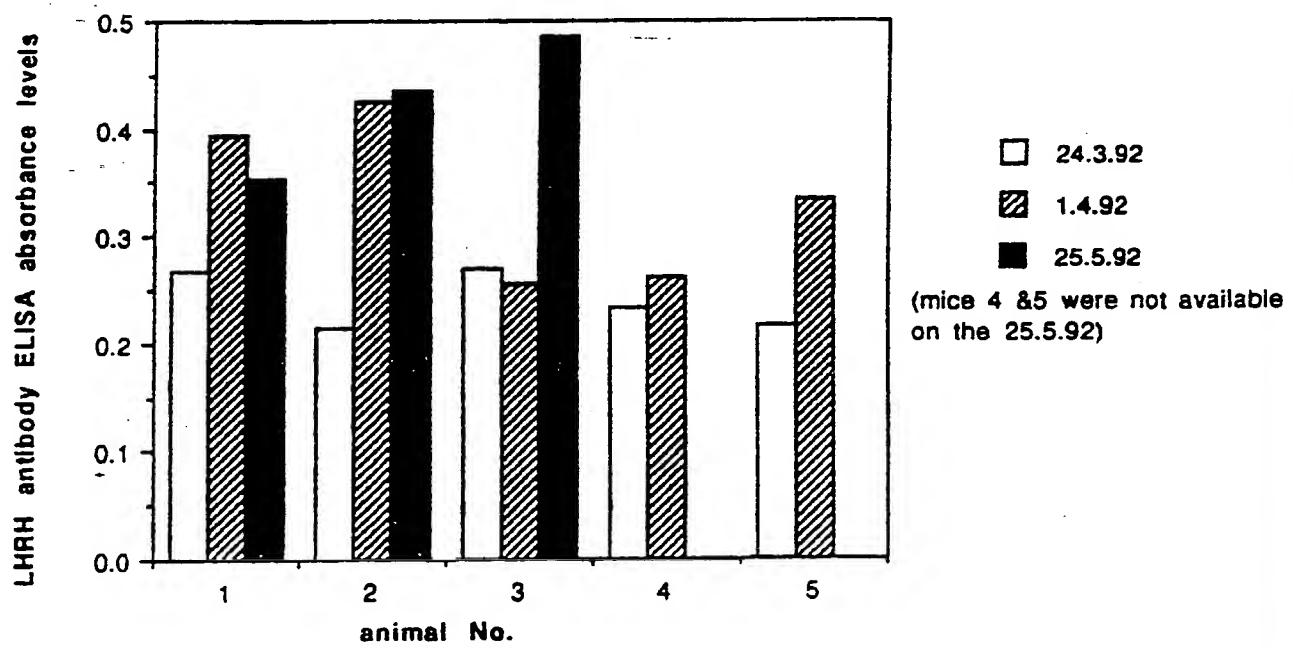


FIG. 8

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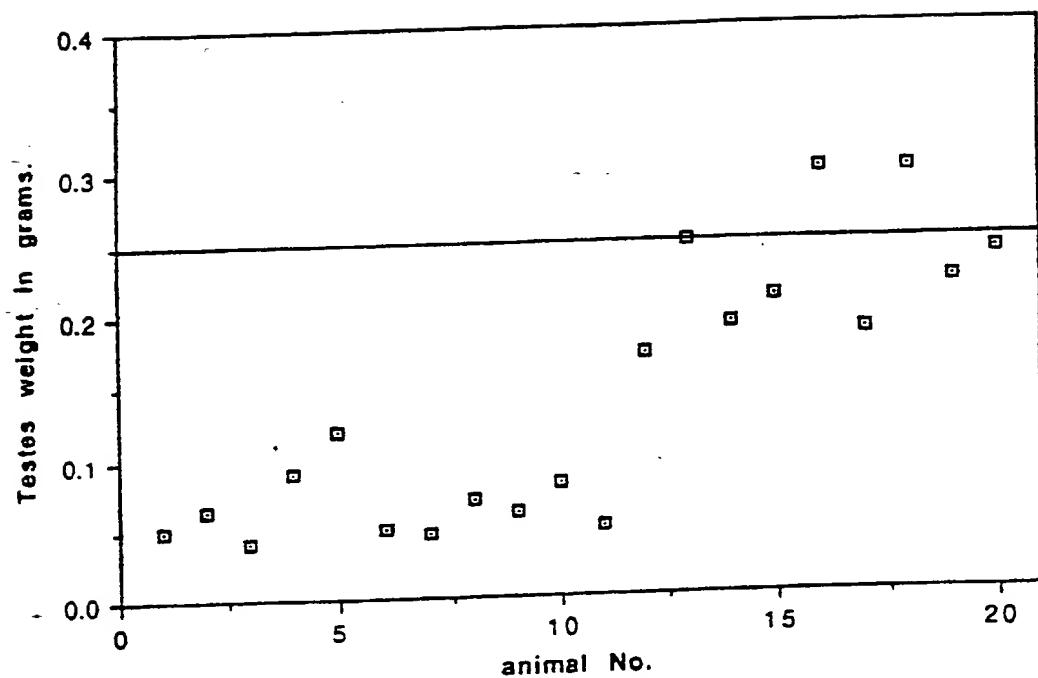


FIG. 9a

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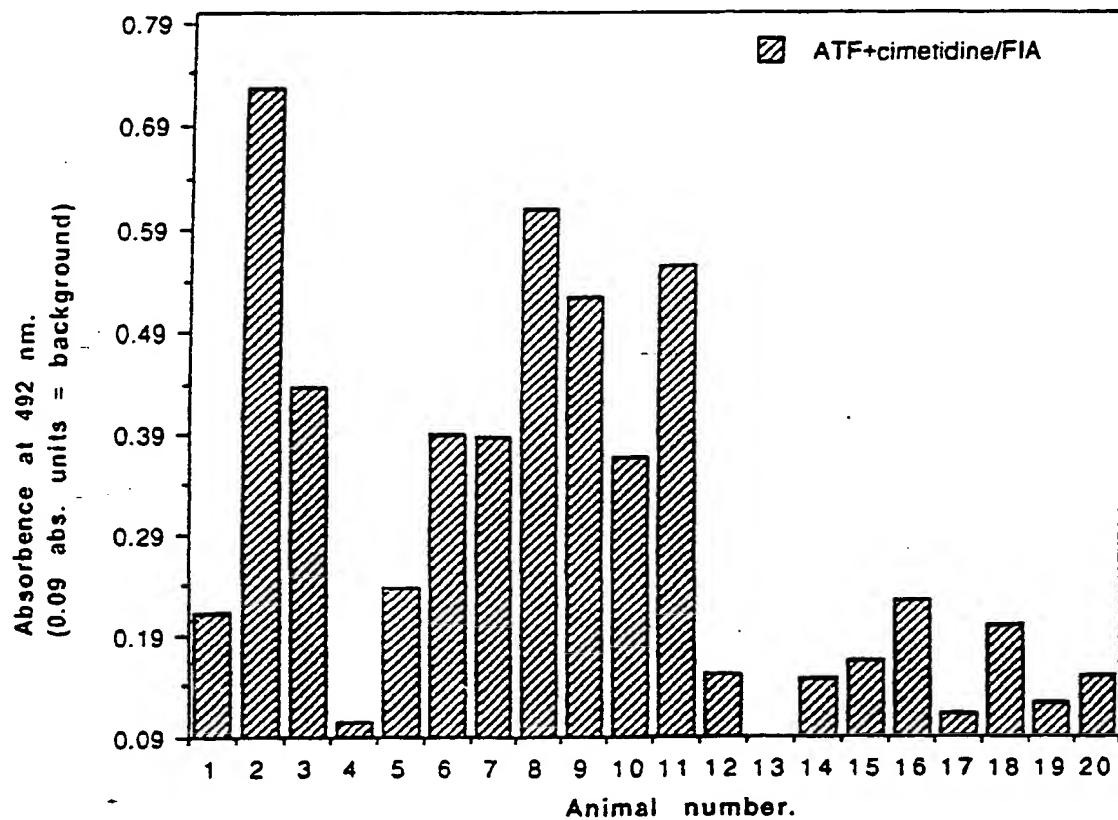


FIG. 9b

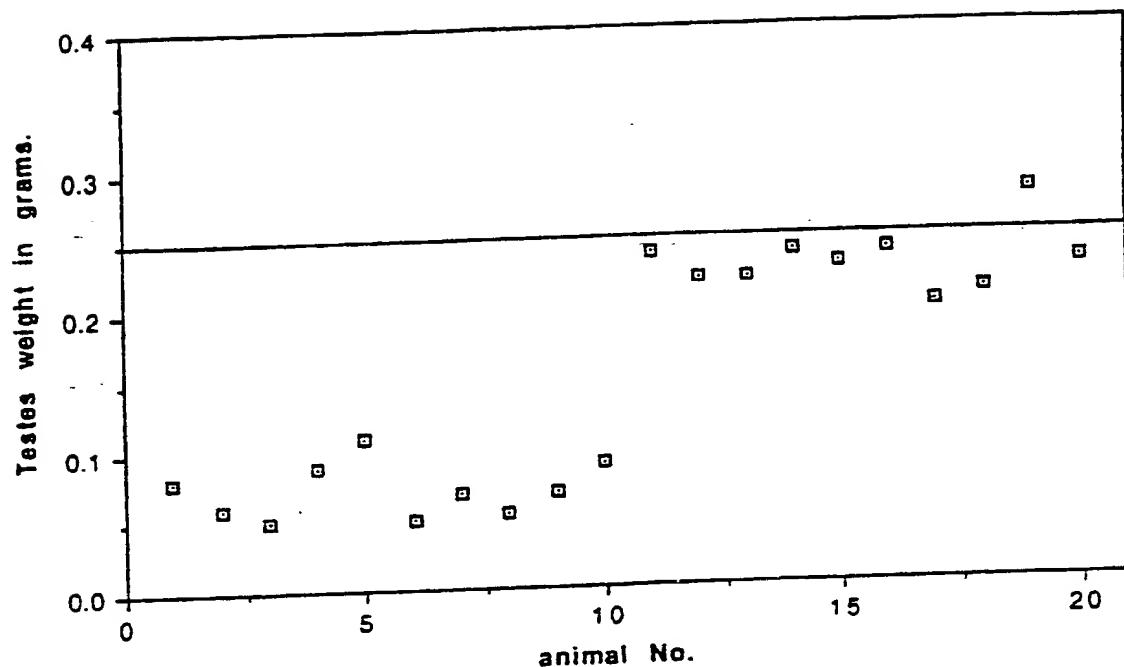


FIG. 10a

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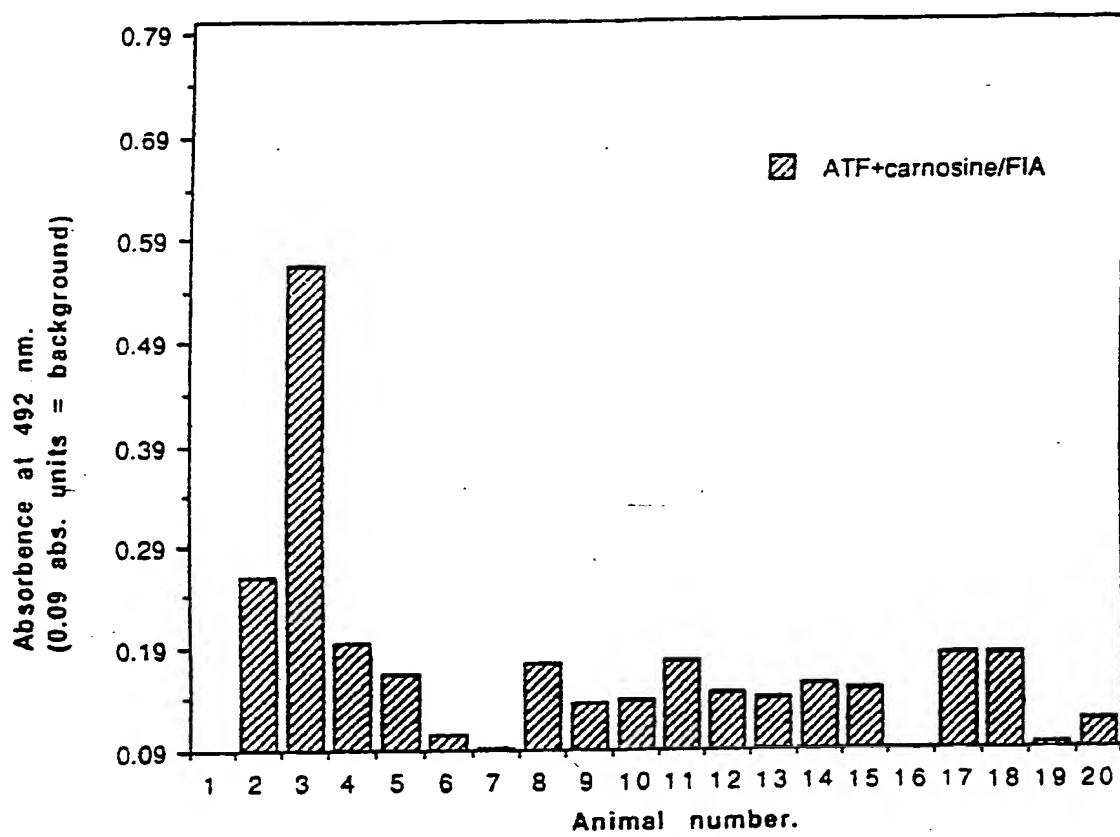


FIG. 10b

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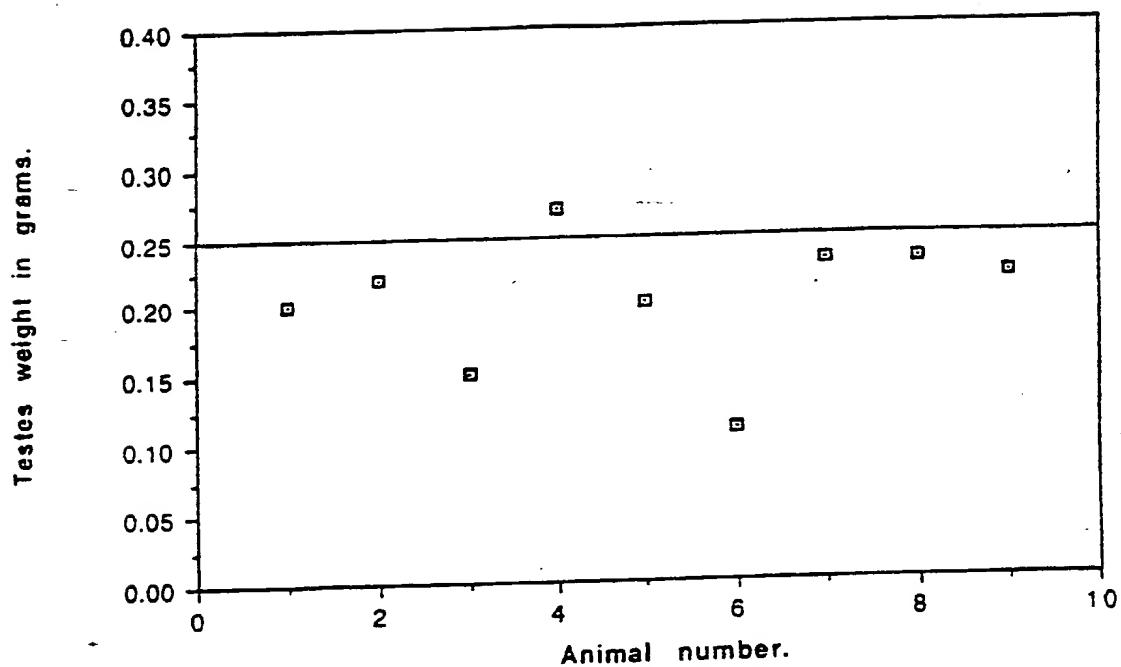


FIG. 11a

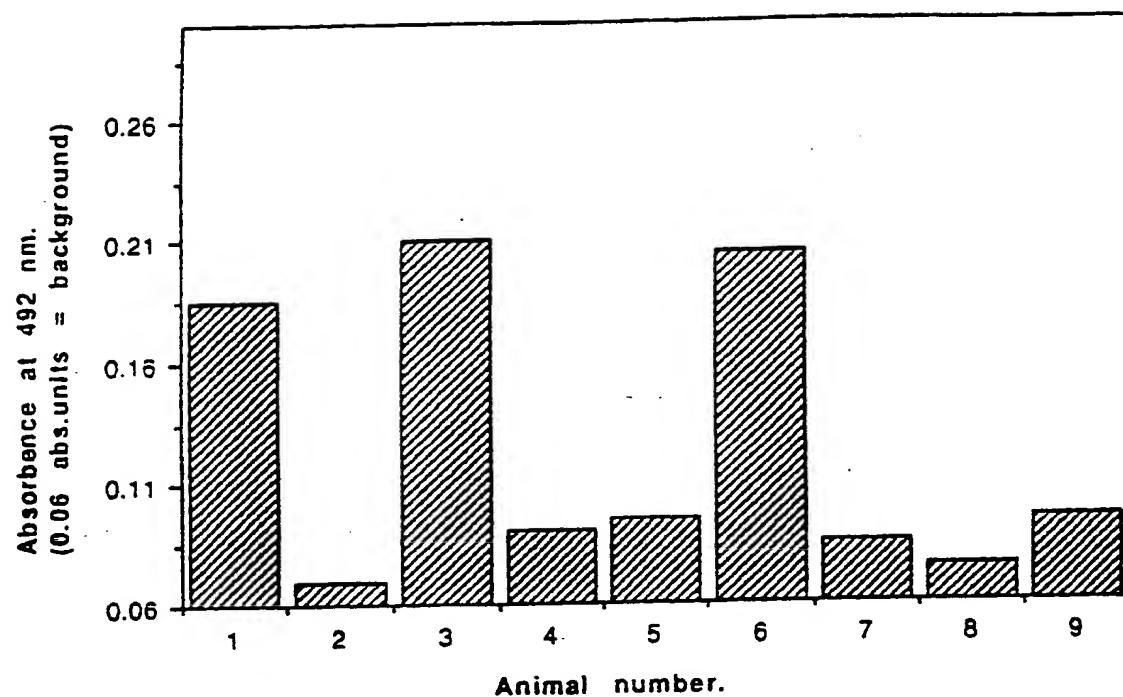


FIG. 11b

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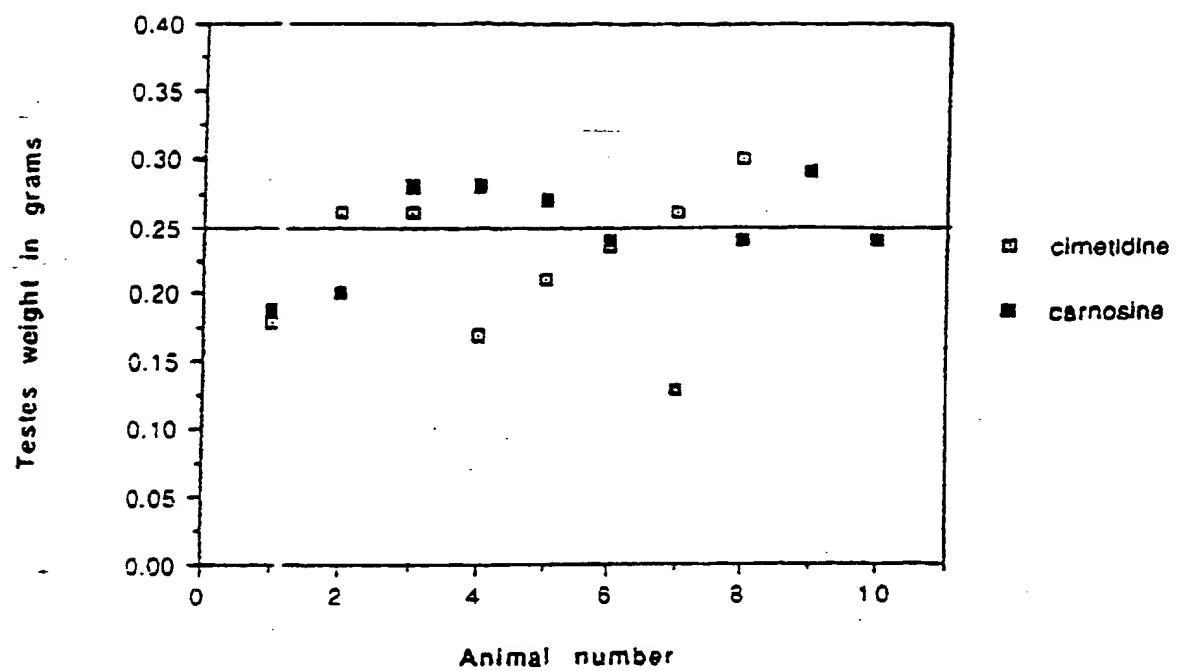


FIG. 12a

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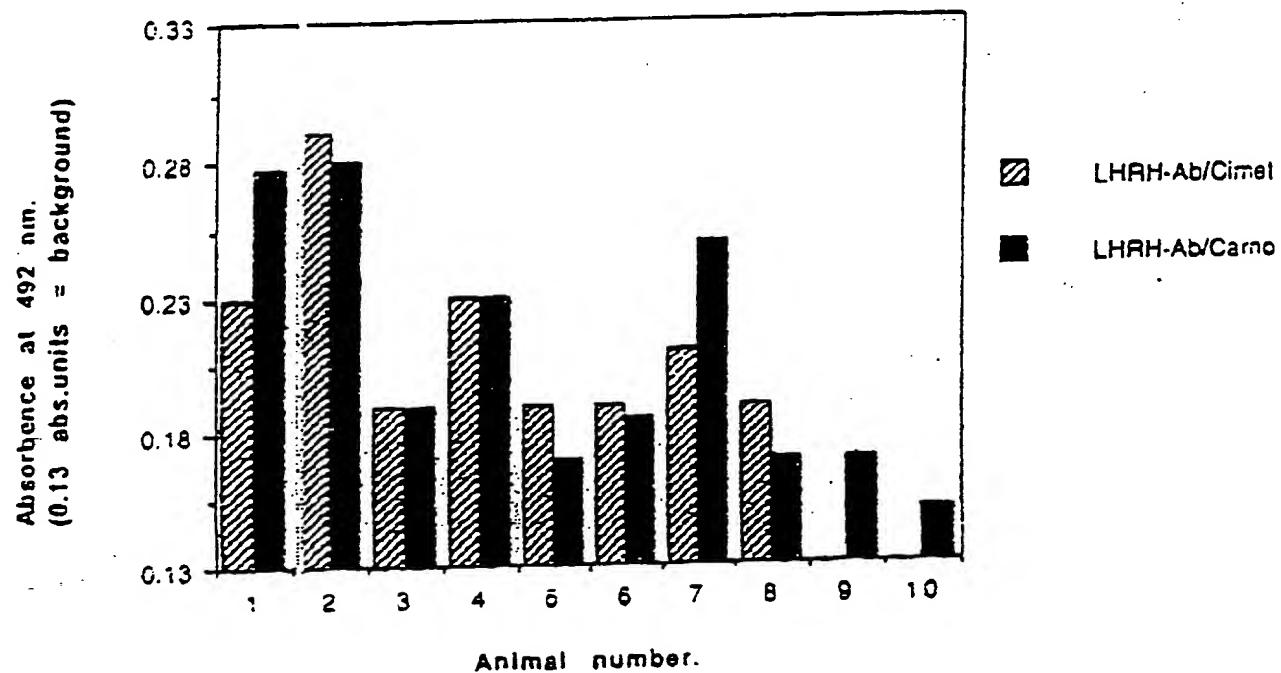


FIG. 12b

**A. CLASSIFICATION OF SUBJECT MATTER**  
 Int. Cl.<sup>5</sup> I.P.C.<sup>5</sup> A61K 39/39, A61K 30/00, A61K 39/02

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
 I.P.C.<sup>5</sup> A61K 39/39, A61K 37/43

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)  
 DERWENT: KEYWORDS: LHRH OR PSEUDOMON OR (TYPE04 AND FIMBIA); ADJUVANT  
 CHEM. ABS: KEYWORDS: AS ABOVE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	AU,A,17049/88 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION) 3 November 1988 (03.11.88). See pages 16-18.	1-4,6,17,20
Y	AU,A,11017/88 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION) 10 August 1988 (10.08.88). See pages 11-33, claims 5-7,11	1-2,6,18
Y	AU,A,77584/87 (BUNGE (AUSTRALIA) PTY LTD) 25 February 1988 (25.02.88). See whole document.	1-2,6,18

Further documents are listed in the continuation of Box C.

See patent family annex.

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 "X" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art  
 "Y" document member of the same patent family  
 "&"

Date of the actual completion of the international search  
 30 October 1992 (30.10.92)

Date of mailing of the international search report

5 Nov 1992 (05.11.92)

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AUSTRALIAN PATENT OFFICE  
 PO BOX 200  
 WODEN ACT 2606  
 AUSTRALIA

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
E,Y	DE,A1,4003944 (PRINZHAUS, GERHARD) EARLIEST PRIORITY DATE 14 FEBRUARY 1990 (14.02.90) See abstract	1-2,6,19
Y	AU,A,42186/89 (PITMAN-MOORE, INC) 5 April 1990 (05.4.90) See pages 12-14	1-2,6,19
Y	US 5017558 (VYAS) 21 May 1991 (21.06.91). See column 6, lines 49-58, column 6, lines 68 to column 7, line 5	1-2,6,17
Y	AU,A,3446189 (SYNPHARM LTD AND SANTER, VIVIEN, BEDFORD) 2 November 1989 (02.11.89) See column 5, page 6, claim 4.	1-2,6,17-20
Y	AU,A,22755/88 (UNIVERSITY OF SASKATCHEWAN) 20 April 1989 (20.04.89). See examples.	1-2,6,18

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report			Patent Family Member				
AU	17049/88	WO	8808430	DE	8805565	EP	291194
		FR	2614423	IT	8853100	JP	1503174
AU	11017/88	WO	8805308	ZA	8800149		
AU	77584/87	EP	282501	JP	1500663	WO	8801176
DE	4003944						
AU	42186/89	EP	437450	JP	4503663	NZ	230820
		US	5036047	WO	9003182		
US	5017558						
AU	34461/89	DK	2501/90	EP	413709	GB	2217319
		WO	8910348				
AU	22755/88	AT	74516	DE	3869892	DK	5434/88
		EP	309863	JP	2000113	NZ	226348
		US	4975420	ZA	8807304		

